Microfluidic measurement of microbial bioburden by fluorescent LAL assay.

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The technology to detect extremely low-level signatures of microbial life is equally important in support of Planetary Protection requirements, as well as life detection missions. Furthermore, the ability to report data near the point of collection and in near real-time is highly desirable. The use of microfluidics and other miniaturization technology will allow such portability in hand-held devices and flight instruments. While culture-based methods remain an essential tool, the 2-3 day culture period is excessively time consuming and does not address the level of non-viable organic contamination. During spacecraft assembly, rapid feedback on cleanliness levels may avoid costly delay and reliable *in situ* life detection methods will surely benefit the depth of science investigation capable of being performed on the Moon, Mars and other bodies in our Solar System.

The Limulus Amebocyte Lysate (LAL) assay is a sensitive enzyme cascade that is triggered by microbial cell wall material. Lipopolysaccharide from gram negative bacteria and beta glucan from yeast and mold can be detected as low as 10^{-13} g in less than one hour. The cascade is part of the innate immune system of the horseshoe crab, *Limulus polyphemus* in which microbes that enter its blood system through an open wound are recognized as foreign. Bacterial lipopolysaccharide (LPS), also called endotoxin, is an integral part of the outer membrane and begins the cascade by binding to, and activating the protease pro-enzyme. Techniques to discriminate whole cells from fragmented cells will be discussed. The LAL test has been adapted to use chromogenic or fluorogenic substrates we use to quantify the reaction. We report successful analysis of fluorogenic LAL reactions in a prototype microfluidic chip.

LAL reactions (Endochrome, Charles River Laboratories) were performed on a series of LPS concentration using the fluorogenic substrate Ile-Glu-Gly-Arg-AMC (Enzyme Systems) and standard endpoint analysis. Reactions were read both in a Tecan Spectrafluor Plus microplate fluorometer as well in the microfluidic chip (Caliper 42). The figure below shows the same standard LPS concentration series run in a standard Tecan microtiter plate and in the Caliper 42 microfluidic system. Despite the large difference in path length, both curves show equal sensitivity. We will continue development of the microfluidic LAL to incorporate all aspects of sample handling, dilution and analysis.